

Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: effects of topical glucocorticosteroid treatment

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Airway challenges with allergen are frequently followed by an increase in responsiveness to both specific and unspecific stimuli. This phenomenon in the lower airways has been linked to the presence of a late phase response to allergen challenge. One of the hallmarks of the late phase response is a tissue infiltration of granulocytes, specifically the eosinophils. A hypothesis for the increase in responsiveness has been that local activation of these eosinophils with subsequent release of the toxic granular constituents leads to a decrease in the barrier function of the airway epithelium. The aim of the present study was to further explore this hypothesis. Ten patients with previously demonstrated nasal allergen-induced hyperresponsiveness participated in a double-blind cross-over study involving pretreatment for various periods with topical glucocorticosteroids. The patients were challenged intranasally with allergen on one day, rechallenge 24 h later and the increase in responsiveness noted. In a nasal lavage performed immediately prior to challenge the number of eosinophilic granulocytes and the level of eosinophilic cationic protein (ECP) was determined. As expected, the placebo-treated patients demonstrated an increase in nasal responsiveness at rechallenge accompanied by a tendency for an increase in eosinophils as well as ECP in the lavage preceding the rechallenge compared with the initial allergen challenge 24 h previously. The amount of ECP found in the lavage on the second day correlated with the TAME-esterase activity, a marker of ongoing inflammatory activity ($r = 0.64$; $P < 0.05$) in the same lavage, suggesting participation of the eosinophilic granulocyte in the inflammatory response to allergen. However, there were no correlations between number of eosinophils or ECP content in the nasal lavages on the first or second day and the degree of the increased responsiveness obtained on the second day. Further, topical treatment with glucocorticosteroids, which totally abolished the increased responsiveness, did not appear to significantly change the levels of ECP in the nasal lavage prior to the initial or re-challenge. It is therefore suggested that the increase in responsiveness seen after an airway allergen challenge is not only a result of eosinophil activation but is a more complex phenomenon.

Key words: eosinophils; eosinophilic cationic protein; hay fever; hyperresponsiveness.

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Hyperresponsiveness of the airways appears to be one crucial factor which contributes to the pathophysiology of allergic airway disease (16). One laboratory model for airway hyperreactivity is the hyperresponsiveness which commonly follows an airway challenge with a pollen aller-

gen, when the challenge is performed outside the pollen season in question. This has been performed in both the lower and upper airways (10, 11, 26). This phenomenon however, appears to be absent in the skin since studies have noted no increase in local dermal responsive-

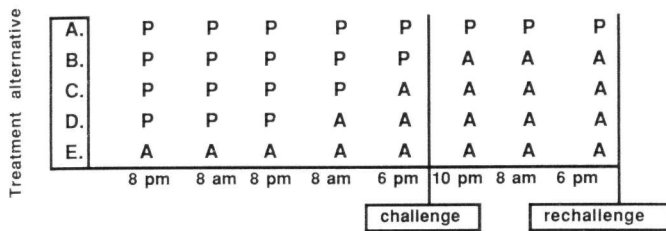


Fig. 1. Schematic outline of treatment schedule in relation to allergen challenges on days 1 and 2. The five treatment alternatives are outlined. A = treatment occasion with active budesonide; P = treatment occasion with placebo spray.

ness following a dermal allergic reaction, regardless of the presence of late phase or allergen-induced airway hyperreactivity in the same individuals (4, 15). It thus appears that the allergen-induced hyperresponsiveness is a phenomenon associated with airways mucosa.

Using a nasal model for induction of hyperreactivity it has been shown that allergen-induced hyperreactivity is associated with an increase in the generation of inflammatory mediators. Further, it has been shown that allergen-induced hyperreactivity is an unspecific phenomenon and not necessarily linked to the size of initiating immediate allergic reaction (1, 2, 3, 26). As in the clinical diseases of asthma and allergic rhinitis, hyperreactivity is highly susceptible to corticosteroid treatment (3, 25, 26). Taken together, these findings implicate the barrier function of the epithelium as one crucial part of allergen-induced hyperreactivity. Although the pathophysiology behind allergen-induced hyperreactivity is not entirely clarified, it has been suggested that the eosinophil plays a crucial role in the development of hyperresponsiveness (12, 21). The basis for this suggestion is that there is an increase in the number of eosinophils on the surface of the nasal epithelium in a time relationship to the late phase of the allergic reaction (5, 6) which in turn, at least in the lower airways, appears to be linked to the allergen-induced hyperreactivity (10). Further, the eosinophils, when stimulated, are capable of releasing highly toxic granular constituents, such as major basic protein (MBP) and eosinophil cationic protein (ECP) which have been shown able to disrupt the integrity of the epithelial lining of the airways (12, 14).

The aim of the present study was to further evaluate the role of the eosinophil and products in the development of allergen-induced hyperreactivity and the pharmacological inhibition of such events by topical glucocorticosteroids.

MATERIAL AND METHODS

The study was part of a double blind randomized, placebo controlled, cross-over study. Nasal challenge and rechallenge were performed after five treatment alternatives: one with placebo and four with topical glucocorticosteroids for different durations (3). A 3-week wash out period was instituted between the five challenge occasions. The study was performed in the pollen-free winter months. The results from other parts have previously been reported (3).

We recruited 10 patients (4 males and 6 females) 20 to 34 years of age (mean age 24.7), with strictly seasonal allergic rhinitis due to birch (*Betula verrucosa*) and/or timothy (*Phleum pratense*). All patients had a positive skin prick test ($\geq$ the histamine control) for the relevant pollen allergen and were asymptomatic at the time of the trial. They had also previously demonstrated nasal hyperresponsiveness in a screening procedure, using the same protocol as in the above study, with at least a two-fold increase in the TAME-esterase activity in the lavage fluid at nasal rechallenge after 24 h compared with an initial challenge with the same allergen dose (1). They had been free from any medication for at least 3 weeks prior to entry into the study and no medication other than the test preparation was allowed during the study. The study was conducted according to the Declaration of Helsinki, approved by the Ethics Committee at the University of Lund, and all patients gave informed consent before taking part.

Treatment

Budesonide, a topical glucocorticoid, was used as a nasal aerosol (RhinocortTM, Draco AB, Sweden). 100 µg/nasal cavity was given on each dosing occasion. The drug was applied twice daily, giving a total daily dose of 400 µg which

is the recommended daily dose, effective in the treatment of hay fever (9). Treatment was started 48 h prior to the initial challenge and maintained up to the rechallenge on day 2, when the final dose was given 2 h before rechallenge. Blindness could be maintained throughout using multiple identical locking canisters containing either active drug or placebo for each treatment period. The treatment schedule is outlined in Fig. 1.

Nasal challenge procedure

The technique used for the nasal challenge has previously been described in detail (1, 3, 22, 23). Briefly, the protocol involved serial lavages (10 min interval) with saline solution to harvest cell free mediators and possible cells floating on the surface of the nasal epithelium. At 10-min intervals, immediately after the saline lavages, the following challenges were performed: oxymethazoline-hydrochloride (to maintain nasal patency), two diluent challenges and then a serial challenge of three doses of allergen, increasing 10-fold (100, 1,000 and 10,000 biolog-

ical units (BU) of birch or timothy allergen solution, PharmalgenTM (Pharmacia, Uppsala, Sweden). The oxymethazoline-chloride solution (0.5 mg/ml) was delivered into each nasal cavity with a mechanical pump spray delivering 50 µl/actuation as 2 actuations into each cavity. The diluent and allergen solution were administered into one nasal cavity with the help of a similar mechanical pump spray delivering 50 µl/actuation. The dose was 2 actuations in the same nasal cavity. The same protocol was used for the rechallenge after 24 h, except that only one allergen dose, the lowest one from the previous day (100 BU) was used. A detailed symptom score sheet was used during the challenge procedure. A composite nasal symptom score was then calculated which included the sum of the scores of blockage and secretion (a five-point scale from 0 to 4 = no symptoms and 4 = severe symptoms) and a sneezing score (0 sneezes = 0, 1-4 = 1, 5-9 = 2, ≥ 10 = 3).

The nasal lavage fluids obtained during the challenge procedure were chilled, centrifuged and the supernatant collected and frozen at -30°C for subsequent TAME analyses, using an assay which has previously been described in detail (19). The first (prechallenge) lavages on

Table 1

ECP levels in nasal lavage fluid obtained before an initial allergen challenge (day 1) and before a rechallenge after 24 h. Values are given after placebo pretreatment and after various pretreatment durations with a topical glucocorticoid (budesonide) prior to the initial allergen challenge. The change from day 1 to day 2 is not statistically significant for any of the treatment alternatives

Treatment start in relation to initial challenge (day 1)	ECP-levels (ng)			
	Mean±SEM (range)		Median	
	day 1	day 2	day 1	day 2
A. Placebo treatment	486±224 (33-2272)	1577±813 (16.5-8325)	102	95
B. 2 h after	136±28 (46.5-256)	295±144 (20-1567)	92	84
C. 2 h before	894±355 (30-2884)	472±132 (72-1237)	124	189
D. 12 h before	101±39 (26-387)	229±76 (38-760)	59	95
E. 48 h before	133±27 (6-256)	306±141 (37-1485)	41	84

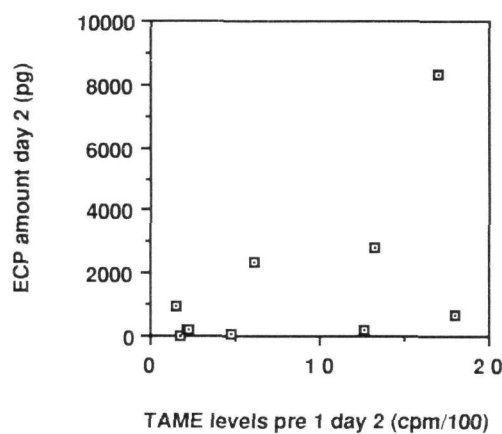


Fig. 2. Relationship between the amount of ECP in the first lavage on day 2 and the TAME-esterase activity in the same lavage. A weak, although statistically significant, correlation was found ($r = 0.64$; $P < 0.05$).

days 1 and 2 were also used for cell and ECP analysis (see below).

Cell analysis. In the first (prechallenge) lavage on each day the centrifuged cell pellet was re-suspended in 1 ml of ringer solution, and 200 μ l aliquots loaded on a cytocentrifuge. After fixation and staining with eosin-hemotoxylin the total number of eosinophils found on the glass slide was counted. The number thus obtained represented a semiquantitative number allowing comparisons between the different challenge days.

ECP-analysis. The level of ECP in the first (pre-challenge) lavage on day 1 and day 2 were

analysed with a radioimmunoassay previously described in detail (30).

Statistical analysis. The statistical evaluation was performed on a microcomputer (Macintosh, Apple Computer, Cupertino, USA) with the aid of a statistical software package (Statview 512+ by Brainpower Inc, Calabassas, CA, USA). A Kruskal Wallis (non-parametric analysis of variance) test was performed to see whether there was a difference between any of the treatment alternatives. Wilcoxon's signed rank sum test was used for further analysis and a Spearman rank correlation was utilized. P values < 0.05 were considered significant (two-sided alternative).

RESULTS

All 10 subjects completed the study. The obtained levels of ECP are presented in Table 1, which shows the mean and median values for the 10 different observations. Although there was a tendency for an increase (NS) in ECP levels immediately before the rechallenge during the placebo treatment, a similar trend was noted also for the other active treatment occasions. No changes observed were statistically significant. Further analysis was first made on the placebo-treated occasions. Eosinophils in any countable numbers were present only during the placebo treatment period. A trend towards increase on day 2 as compared with day

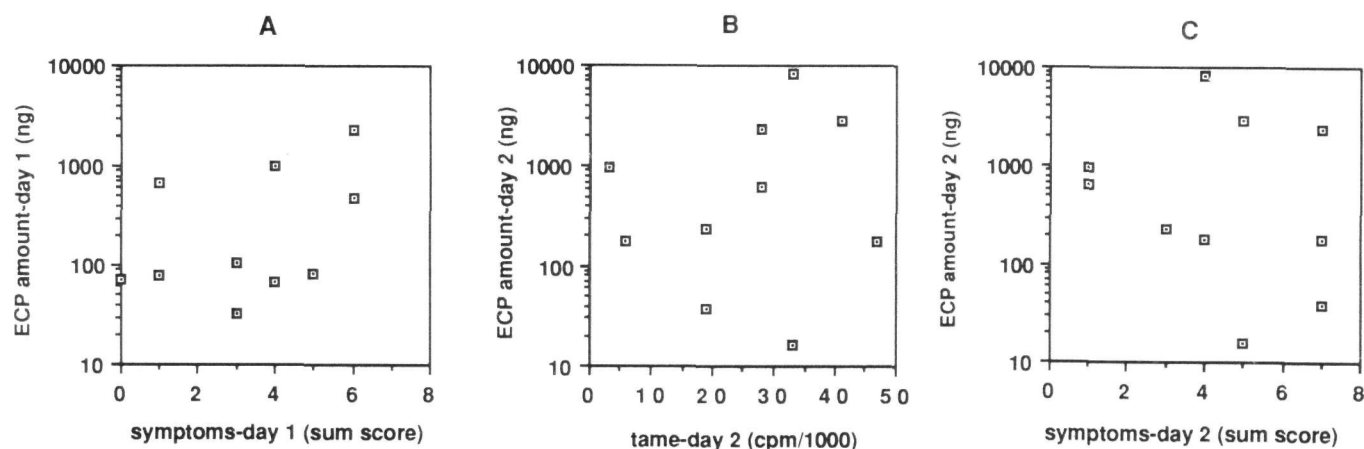


Fig. 3. Composition figure showing the relationship between the amount of ECP harvested and symptoms and TAME-generation following challenge with 100 BU/ml. Panel A, shows the relationship between the ECP amount day 1 and symptoms day 1 ($r = 0.47$; NS). Panel B shows the relationship between the ECP amount day 2 and TAME generation day 2 ($r = 0.01$; NS). Panel C shows relationship between the ECP amount day 2 and symptoms day 2 ($r = 0.27$; NS).

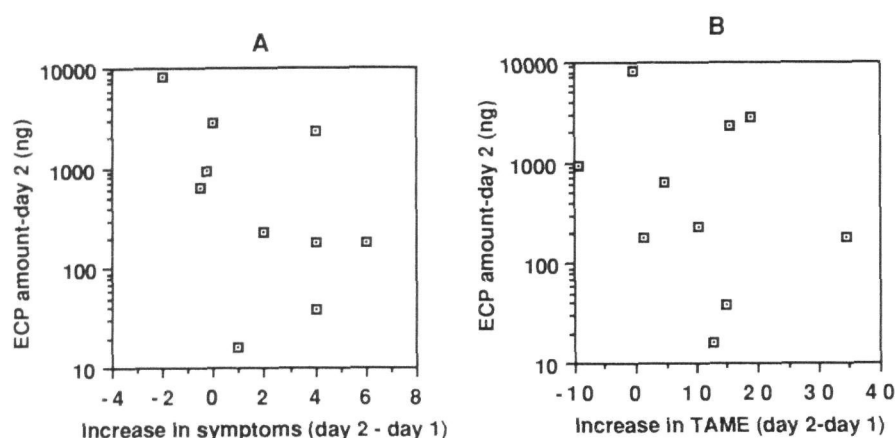


Fig. 4. Composite figure showing the relationship between the amount of ECP harvested on day 2 and the increase in symptoms and TAME-generation from day 1 to day 2 following challenge with 100 BU/ml. Panel A shows the increase in symptoms ($r = 0.26$; NS) and panel B the increase in TAME-generation ($r = .57$; NS).

1 was noted, with a median value of 0.5 on day 1 compared with a median value of 1.5 on day 2. However, there was no correlation between the number of eosinophils present and the ECP levels obtained on any of the two observation days. To further elucidate the role of ECP in putative ongoing inflammatory reactions after the allergen challenge, a correlation was made between the TAME-esterase activity in the first lavage on day 2 and the ECP levels in the same lavage. There was a statistically significant correlation between these two parameters ($r = 0.64$; $P < 0.05$) as shown in Fig. 2. The role of ECP in the allergen-induced hyperreactivity appears less clear. No correlations were observed between the symptoms and TAME values obtained at the placebo-treated occasion and the ECP levels obtained (Fig. 3 A-C). This was true for the ECP levels that preceded the allergen challenge (100 BU) on the first day (Fig. 3 A) and the ECP levels obtained on the second day that preceded the symptom obtained at the rechallenge with allergen (100 BU) (Fig. 3 B, C). Fig. 4 A, B shows there were no obvious correlations between increases in nasal symptoms and the TAME-generation from day 1 to day 2 and the levels of ECP in the first lavage on day 2. Similar lack of correlations was obtained for the different active treatment periods (data not shown). None of the different glucocorticoid treatment alternatives induced any statistically significant changes in ECP lev-

els, either on the first or the second day compared with the placebo treatment alternative.

DISCUSSION

The present study demonstrates a relationship between the activation of eosinophils and other signs of allergen-induced inflammation of the allergic nasal mucosa. It also demonstrates a lack of correlation between the presence of a product of eosinophil activation in nasal lavages and the increased local responsiveness seen after a nasal allergen challenge.

In the present study we have chosen to further study the nasal allergen-induced hyperresponsiveness. This feature of the allergic reaction is present in a continuous allergic disease such as hay fever (8, 20) and, like the clinical disease, is highly susceptible to treatment with corticosteroids both orally and topically (3, 25, 26, 28). The change in responsiveness may, at least partly, be due to a change in the barrier function of the epithelium, a crucial factor in the continuation of allergic airways disease (13, 17).

Although there are indications that eosinophilic granulocytes play an active role in the development of airway hyperreactivity, supportive human data are scanty. There are indications that circulating eosinophilic granulocytes are activated during seasonal allergic rhinitis (27, 29). Studies of seasonal allergic

rhinitis have also demonstrated an increase of eosinophilic granulocytes on the surface of the nasal mucosa with a relationship to nasal symptoms (24). Further, nasal challenge experiments have shown not only an increase in eosinophilic granulocytes but also of ECP and MBP in nasal lavages in a time-relationship to a nasal late phase (5, 6, 7). This suggests that activation of these cells with subsequent release of ECP and MBP contribute to the development of nasal allergen-induced hyperreactivity.

Although it has been suggested that allergen-induced hyperreactivity as a feature of allergic mucosal disease is linked to an allergic late phase, the evidence for this apparent link is lacking, at least for allergic reactions in the upper airways (18). Although a previous study pointed to the possibility of a relationship between ECP levels in nasal secretions and nasal-allergen induced hyperreactivity (21), we failed to note such a relationship but found a wide variation of ECP levels with no clearcut relationship to the induction of hyperreactivity and putative glucocorticoid therapy. There may be several reasons for this discrepancy between our study, where there was no relationship to any induced hyperreactivity, and the previous one (21) indicating a relationship. One of the reasons is that in the present study were selected only patients who had previously shown allergen-induced hyperreactivity. It may well be that these patients, as a group, have higher levels of ECP compared with a unselected patient group. Nevertheless, we failed to find any relationship between the degree of allergen-induced responsiveness and the levels of ECP present in the nasal lavages prior to challenge. Further, the treatment with glucocorticosteroids, which completely abolished the nasal allergen-induced hyperresponsiveness, had no significant influence on the levels of ECP.

The lack of any clearcut relationships points to the conclusion that the development of allergen-induced hyperreactivity is much more complicated than being only the recruitment and subsequent local activation of a single cell type with a continuous release of cytotoxic granular constituents that abrogates the barrier function of the airway epithelium.

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